does not need to be exactly regulated, especially those that are constitutively regulated, and those defects which are found in the patient's bone marrow.

For example, one disease candidate for gene therapy is adenosine deaminase (ADA) deficiency which results in severe combined immunodeficiency disease (SCID). ADA deficient patients have little or no detectable enzyme in bone marrow cells. However, ADA deficiency has been cured by matched bone marrow transplantation. ADA normal cells have a selective advantage over ADA deficient cells and will normally repopulate the patient's bone marrow.

Bone marrow cells are a good target for somatic gene therapy because bone marrow tissue is easily manipulated *in vitro* and contains repopulating cells. Alternatively, human cord blood has previously also been demonstrated to contain a large number of primitive progenitor cells. Successful gene transfer into hematopoietic stem cells, the long term repopulating cells, may lead to lifelong cures for a variety of diseases manifested in the progeny of these cells.

Gene transfer into, and long term gene expression in, repopulating stem cells has been achieved in murine models by a number of investigators. However, *in vivo* experiments in larger animals, such as dogs and primates, have met with limited success,

largely due to the low efficiency of infection of primitive hematopoietic stem cells. The limitations of current gene transfer technology are further complicated when applied to human protocols by several factors, including the low numbers of stem cells present in adult bone marrow (ABM), the lack of suitable methods to purify these cells, and the low fraction of such primitive cells in cell cycle.

In both murine and large animal experiments involving bone marrow cells, it has been noted that the most successful protocols utilize cocultivation of target cells with retroviral producer cell lines. Also, most of the FDA- approved gene transfer trials in humans rely on recombinant retroviral vectors for gene transduction. Recombinant retroviral vectors are desirable for gene therapy because they efficiently transfer and precisely and stably integrate exogenous DNA into cellular DNA. These vectors contain exogenous DNA for gene transfer and are further modified to eliminate viral pathogenicity. Because of these modifications, viral production is generally accomplished using retrovirus packaging cells. However, for clinical gene therapy, cell-free transduction is more desirable due to concerns about bio-safety and quality control. Unfortunately, efficient gene transfer into hematopoietic cells such as stem cells has generally not been possible without cocultivation with virus-producing cells.